

# Isolation of a cDNA encoding a photoreceptor cell-specific actin-bundling protein: retinal fascin

Yoshitsugu Saishin<sup>a,b,\*</sup>, Shoichi Shimada<sup>b</sup>, Hiroyuki Morimura<sup>a</sup>, Kohji Sato<sup>b</sup>,  
Ichiro Ishimoto<sup>a</sup>, Yasuo Tano<sup>a</sup>, Masaya Tohyama<sup>b</sup>

<sup>a</sup>Department of Ophthalmology, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan

<sup>b</sup>Department of Anatomy and Neuroscience, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan

Received 5 June 1997; revised version received 28 July 1997

**Abstract** We have isolated a novel retina-specific gene, retinal fascin, encoding a new member of actin-bundling protein gene family, from a bovine retina cDNA library. The cDNA encodes a 492 amino acid protein which shows 36–57% amino acid identity with three vertebrate fascins, echinoid fascin and *Drosophila* singed gene. Northern blot analysis revealed that retinal fascin mRNA was exclusively expressed in the eye and not seen in other tissues examined. In situ hybridization analysis indicated that retinal fascin mRNA signals were found only in the inner segment of the photoreceptor layer and outer nuclear layer, indicating that retinal fascin was specifically expressed in photoreceptor cells. As fascins are actin-bundling proteins important for constructing several intracellular structures, retinal fascin might play a pivotal role in photoreceptor cell-specific events, such as disk morphogenesis.

© 1997 Federation of European Biochemical Societies.

**Key words:** Retinal fascin; Actin-bundling protein; Photoreceptor cell; Retina-specific gene; cDNA cloning

## 1. Introduction

Fascins are a widely distributed family of proteins that organize filamentous actin into bundles [1]. Kane first isolated echinoid fascin from cytoplasmic extracts of sea urchin eggs, which is required for producing a highly organized gel consisting of needle like structures [2,3]. The cDNA for echinoid fascin has been isolated subsequently, revealing that echinoid fascin shows homology with the *Drosophila* singed gene product responsible for gnarled bristle development and female sterility [4]. To date, members of fascin gene family have been also cloned from human, mouse and *Xenopus* [5–7]. Each of these proteins functions as an actin-bundling protein in many structures, such as microspikes and stress fibers of fibroblasts, filopodia of coelomocytes, bristles and nurse cells of *Drosophila* [1]. Their wide distribution suggests that fascins play important roles in morphogenesis of diverse intracellular structures.

In photoreceptor cells, actin is known to localize in the inner segment [8–12], synaptic region of rods [11–13], the connection between rod inner and outer segments [10,11], the lip of forming rod outer segment discs [10,14–16] and seems to be responsible for many functions, such as regulation of outer segment disk morphogenesis [18] and photoreceptor shortening during light or dark adaptation in lower vertebrates [19,20]. And these special functions of actin in photoreceptor

cells raise the possibility that these cells might express their own actin-bundling proteins for regulating such structures. However, almost no information is available on actin-bundling proteins in the retina. In the course of our attempt to isolate retina-specific genes, we cloned a novel member of fascin gene family, retinal fascin, whose transcripts were exclusively expressed in photoreceptor cells. In the present study, we report its primary structure and expression pattern in the retina.

## 2. Materials and methods

### 2.1. cDNA library screening

A bovine retina cDNA library constructed in the Uni-ZAP XR vector (Stratagene) was screened by random primed labeling with [<sup>32</sup>P]dCTP. The probe was made from one of the clones randomly picked up from retina cDNA library and showing an eye-specific expression pattern by Northern blot analysis. Probes were hybridized to the phage DNA immobilized on nylon membranes (Hybond-N+, Amersham Corp.) in a solution containing 5×SSPE, 2×Denhardt's solution, 0.1% SDS, 40% formamide, 50 µg/ml denatured salmon sperm DNA. They were incubated at 37°C for 20 h and washed 2 times at 55°C each time in 1×SSPE, 0.4% SDS. The membranes were exposed to autoradiographic film (X-Omat AR, Eastman Kodak Co.) in cassettes overnight at room temperature. After second screening, positive clones were excised in vivo with R408 helper phage. We subcloned the cDNA inserts into pBluescript II SK(+).

### 2.2. DNA sequencing

DNA sequencing was performed on the dideoxy chain termination method (Applied Biosystems Inc.) using Ampli Taq DNA polymerase. It was analyzed with an automatic DNA sequencer (ABI). DNA sequencing was confirmed by both strands.

### 2.3. Northern blot analysis

mRNA was isolated from various rat organs with a Fast Track mRNA purification kit (Invitrogen). 3 µg of mRNA was electrophoresed in a 1.0% agarose formaldehyde gel and transferred onto a nylon membrane (Hybond-N+, Amersham Corp.). Partial clones of rat retinal fascin and rat GAPDH cDNA were labeled with [<sup>32</sup>P]dCTP (4500 Ci/mmol, ICN) using a random primer DNA labeling kit (Takara). Hybridization was performed at 42°C overnight in a solution containing 5×SSPE, 5×Denhardt's solution, 1% SDS, 50% formamide, 200 µg/ml denatured salmon sperm DNA. The membrane was washed 2 times in 2×SSPE and 0.1% SDS at room temperature for 10 min and washed in 1×SSPE and 0.1% SDS at 65°C for 15 min and then washed in 0.1×SSPE and 0.1% SDS at 65°C for 10 min. The hybridization signals were analyzed with a bioimaging analyzer BAS 5000 (FUJIX, Tokyo, Japan).

### 2.4. In situ hybridization

The procedures were basically the same as those described previously [21]. Male wistar rats (150 g) were used in this study. In situ hybridization with [<sup>35</sup>S]UTP-labeled cRNA probes transcribed from partial rat retinal fascin cDNA was performed on retina sections from adult rats.

\*Corresponding author. Fax: +81 (6) 879-3229.  
E-mail: saishin@anat2.med.osaka-u.ac.jp

A	5'	AGG GGC CCC CGT CGG GGA CCT CAG CCA GGC CCG AAG ATG CCT ACA AAT GGC CTG	54
		M P T N G L	
		CAC CAG GTG CTG AAA ATC CAG TTT GGC CTC GTC AAT GAC ACT GAC CGC TAC CTG	108
		H Q V L K I Q F G L V N D T D R Y L	
		ACG GCC GAG AGC TTT GGC TTC AAG GTC AAT GCC TCA GCA CCC AGC CTC AAG CGG	162
		T A E S F G F K V N A S A P S L K R	
		AAG CAG ATG TGG GTG CTG GAG CCG GAC CCA GGG GAG GGC ACT GCC GTG CTG TTT	216
		K Q M W V L E P D P G E G T A V L F	
		CGC AGC AGC CAC CTG GGC CGT TAC CTG TCG GCC GAG GAG GAC GGG CGT GTG GCC	270
		R S S H L G R Y L S A E E D G R V A	
		TGC GAG GCG GAG CGG CCG GGT CGT GAC TGC CGC TTC CTG GTC CTG CCG CAG CCC	324
		C E A E R P G R D C R F L V L P Q P	
		GAT GGG CGC TGG GTG CTG CAG TCG GAA CCG CAC GGC CGC TTC TTT GGT GGC ACC	378
		D G R W V L Q S E P H G R F F G G T	
		GAG GAC CAG CTG TCC TGC TTC GCC ACG GCC ATC ACC CCA GCC GAG CTG TGG ACA	432
		E D Q L S C F A T A I T P A E L W T	
		GTG CAC CTG GCC ATC CAC CCG CAG GCC CAC CTG CTG AGC GTG AGC CGG CGG CGC	486
		V H L A I H P Q A H L L S V S R R R	
		TAC GCA CAC CTA TGC CCG CAG GAA GAT GAG ATC GCA GCG GAC AGC AAT ACG CCA	540
		Y A H L C P Q E D E I A A D S N T P	
		TGG GGT GTG GAC GCG CTT GTC ACG CTC ATC TTC CAG AAC CGG CAG TAC TGC CTC	594
		W G V D A L V T L I F Q N R Q Y C L	
		AAG TCC TGT GAC AGC CGC TAC CTG CGC AGC GAC GGC CGC CTC GTC TGG GAG CCC	648
		K S C D S R Y L R S D G R L V W E P	
		GAG GCT CGT GCC CGC TAC ACG CTT GAG TTC AAG GCG GGC AAG TTG GCC TTC AAG	702
		E A R A R Y T L E F K A G K L A F K	
		GAC TGC GAT GGC CAC TAC CTG GCA CCC GTG GGC CCC GCG GGC ACG CTC AGG GCG	756
		D C D G H Y L A P V G P A G T L R A	
		GGC CGC AAC ACA CGG CCT GGC AAG GAC GAG CTC TTC GAC CTG GAG GAG AGT CAC	810
		G R N T R P G K D E L F D L E E S H	
		CCA CAG GTG GTG CTG GTG GCC GCC AAC CAC CGC TAC GTG TCC GTG CGG CAA GGG	864
		P Q V V L V A A N H R Y V S V R Q G	
		GTC AAT GTC TCA GCC AAC CAA GAT GAA GAA CTG GAT CAC GAG ACC TTC TTG ATG	918
		V N V S A N Q D E E L D H E T F L M	
		CAA ATT GAC CAG GAG ACA AAG AAG TGC ACC TTC TAT TCC AGC ACT GGG GGC TAC	972
		Q I D Q E T K K C T F Y S S T G G Y	
		TGG ACC CTG GTC ACC CAC GGG GGC ATC CAG GCC ACA GCT ACA CAA GTT TCT GAG	1026
		W T L V T H G G I Q A T A T Q V S E	
		AAC ACC ATG TTT GAA ATG GAG TGG CGG GGC CGA CGG GTG GCC CTC AAG GCC AGT	1080
		N T M F E M E W R G R R V A L K A S	
		AAC GGG CGC TAT GTG TGC ATG AAG AAG AAT GGG CAG CTG GCG GCC ATC AGC GAT	1134
		N G R Y V C M K K N G Q L A A I S D	
		TTT GTG GGG GAG GAC GAG GAG TTC ACG CTC AAG CTT ATC AAC CGG CCC ATC CTG	1188
		F V G E D E E F T L K L I N R P I L	
		GTC CTG CGC GGC CTG GAC GGC TTC GTC TGC CAC CGA CGT GGC TCC AAC CAG TTG	1242
		V L R G L D G F V C H R R G S N Q L	
		GAC ACC AAC CGC TCG GTT TAC GAC GTG TTC CAC CTG AGC TTC AGC GAC GGC GCC	1296
		D T N R S V Y D V F H L S F S D G A	
		TAC CAG ATC CGA GGC CGC GGC GGC TTC TGG CAC ACC GGC AGC CAC GGC AGC	1350
		Y Q I R G R G G G F W H T G S H G S	
		GTG TGC AGC GAC GGC GAG CGC GCC GAG GAC TTC CTG TTC GAG TTC CGG GAG CGC	1404
		V C S D G E R A E D F L F E F R E R	
		GGC CGC CTG GCC ATC CGG GCC CGG AGC GGC AAG TAC CTG CGT GGC GGC GCC TCG	1458
		G R L A I R A R S G K Y L R G G A S	
		GGG CTG CTG CGC GCG GAC GCG GCG CCG GCT GGG GTT GCG CTC TGG GAA TAC	1512
		G L L R A D A D A P A G V A L W E Y	
		TGA GCG CCG CCC GTC TGT CCC CCA TTA AAC CGT GTC TGT GAA AAA AAA AAA	1566
		*	
		AAA AAA AAA AAA AAA AAA AAA AA 3'	

Fig. 1. A: Nucleotide and deduced amino acid sequence of retinal fascin. The translation termination codon is marked by an asterisk. This clone contains a cDNA insert of 1589 base pair with an open reading frame encoding a 492 amino acid protein. B: Alignment of amino acid sequences encoding retinal fascin, *Xenopus* fascin, human fascin, murine fascin, echnoid fascin and *Drosophila* singed gene. Dots indicate amino acids conserved in fascin gene family. C: Partial nucleotide sequence of rat retinal fascin cDNA.

<b>B</b>	retinal fascin	1	MPTNGLHQVL	KIQF-----	-----	---GLVNDTD	RYLTAESFGF	50
	Xenopus	1	MSSGP-----	-LQL-----	-----	---GLVNCNN	KYLTAFAFGF	50
	human	1	MTANGTAEAV	QIQF-----	-----	---GLINCGN	KYLTAFAFGF	50
	murine	1	MTANGTAEAV	QIQF-----	-----	---GLISCGN	KYLTAFAFGF	50
	echinoid	1	MPAMN-----	-LKY-----	-----	-KFGLVNSAG	RYLTAEFKGC	50
	Drosophila	1	MNGQG-----	-CELGHNSGD	IISQNNQKQW	WTIGLINGQH	KYMTAETFGF	50
			*			**	* * * * *	
	retinal fascin	51	KVNASAPSLK	RKQMWVLEPD	PGEGT-AVLF	RSSHLLGRYLS	AEDDGRVACE	100
	Xenopus	51	KINASASSLK	KKQVWSLEPA	GDDT--SAVL	LRSHLGRFLS	ADKDGKVSGE	100
	human	51	KVNASASSLK	KKQIWTLEQP	PDEAGSAAVC	LRSHLGRYLA	ADKDGNVTCF	100
	murine	51	KVNASASSLK	KKQIWTLEQP	PDEAGSAAVC	LRTHLGRYLA	ADKDGNVTCF	100
	echinoid	51	KVNASGATLK	ARQVWILEQE	ESS---TISY	LKAPSGNFLS	ADKNGNVYCS	100
	Drosophila	51	KLNANGASLK	KKQLWTLEPS	NTGE--SIY	LRSHLNKLYLS	VQDFGNVLCF	100
			* * *	* * *		*	* * *	
	retinal fascin	101	AERPRG--DC	RFLVLP--QP	DGRWVLQSEP	HGRFFGGTED	QLSCFATAIT	150
	Xenopus	101	SD--TAGPEC	RFLVSA--QG	DGRWALQSEL	FGRYFGCSSE	RISCFSPSVS	150
	human	101	RE--VPGPDC	RFLIVA--HD	DGRWSLQSEA	HRRYFGGTED	RLSCFAQTVS	150
	murine	101	RE--VPDGDC	RFLVVA--HD	DGRWSLQSEA	HRRYFGGTED	RLSCFAQSVS	150
	echinoid	101	VEDRTEDAT	GFEIEL--QP	DGKWALKNVS	HQRYLACNGE	ELICESSTVS	150
	Drosophila	101	SDERDAGTRG	RFQISISEDG	SGRWALKNES	RCYFLGGTPD	KLVCTAKTPG	150
			*		* * *		*	
	retinal fascin	151	-PAELWTVHL	AIHPQAHLLS	VSRRRYAHLC	PQE---DEI	AADSNTPWGV	200
	Xenopus	151	-PAEKWGVHL	AMHPQFTLYS	VTRKRYRRL-	--SA-SGDEL	SVERDVPWGV	200
	human	151	-PAEKWSVHI	AMHPQVNIYS	VTRKRYAHL-	--SARPADEI	AVDRDVPWGV	200
	murine	151	-PAEKWSVHI	AMHPQVNIYS	VTRKRYAHL-	--SARPADEI	AVDRDVPWGV	200
	echinoid	151	NPSANWTVQL	AIHPQVCMKN	VQHQRVYHLK	TSEE-GEDSV	VVDLVPWGA	200
	Drosophila	151	-ASEFWTVHL	AARPQVNLRS	IGRKRF AHL-	--SE-SQDEI	HVDANTPWGE	200
			* *	* * *	*	*	* * *	
	retinal fascin	201	DALVTILFQN	RQYCLKSCDS	RYLRS---DG	RLVWEPEARA	RYTLEFKAGK	250
	Xenopus	201	DSLITLLFQ-	E--NRYSIQT	PDHRLASDG	SLREKPGPDT	AYTLDSLAKG	250
	human	201	DSLITLAFQ-	D--QRYSVQT	ADHRFLRHOG	RLVARPEPAT	GYTEFRSGK	250
	murine	201	DSLITLAFQ-	D--QRYSVQT	SDHRFLRHOG	RLVARPEPAT	GTFLEFRSGK	250
	echinoid	201	DSTLTLVYLK	K--GKYGLEA	FNGKFVQTDG	QLAGTANEQT	QFTLIFTSGH	250
	Drosophila	201	DTLFTLEFRA	EEGGRYALHT	CNNKYL NANG	KLQVVCNEDC	LFSAEYHGGH	250
			*	* *	*	*	*	
	retinal fascin	251	LAFKDCDGHY	LAPVGPAGTL	RAGRNTTRPK	DELFDLEESH	PQVVLVAANH	300
	Xenopus	251	VAFRGSDGRY	LTSSGPGSGL	KSGKNSKAGR	DELFLVLRSC	PQVVLTAGNG	300
	human	251	VAFRDCEGRY	LAPSGPGSGL	KAGKATKVKG	DELFALEQSC	AQVVLQAANE	300
	murine	251	VAFRDCEGRY	LAPSGPGSGL	KAGKATKVKG	DELFALEQSC	AEVVLQAANE	300
	echinoid	251	LVLDRDNGRH	LGVDSDGTRVL	KSSKPG-LTK	ANYFLEDSC	PQGAFFFG--	300
	Drosophila	251	LALDRDQGGY	LSPIGSKAVL	KSRSS-VTR	DELFSLEDLS	PQASFIAGHN	300
			*	*	*	* * *	*	
	retinal fascin	301	R-YVSVRQGV	NVSAN---QD	EELDHETFLM	QIDQETKKC-	----TFYSST	350
	Xenopus	301	GT-CLQGGV	IFSAN---QD	EESDQETFFQ	EINKDTKMC-	----AFRTHT	350
	human	301	RN-VSTRQGM	DLSAN---QD	EETDQETFFQ	EIDRDTKKC-	----AFRTHT	350
	murine	301	GN-VSTRQGM	DLSAN---QD	EETDQETFFQ	EIDRDTKKC-	----AFRTHT	350
	echinoid	301	GKYASLKQGE	DVSFKLLVDE	DIEDTETFFQ	EFVETDKYAI	RVCDPKNSR	350
	Drosophila	301	LRYVSVKQGV	DVTAN---QD	EVGENETFFQ	EYDWSAHRW-	----ALRTTQ	350
					***			
	retinal fascin	351	GGYWTLVTHG	GIQATATQVS	EN-TMFEMEW	RGRRVALKAS	NGRYVCMKKN	400
	Xenopus	351	GKYWTLSSNG	GIQASASTLN	SS-CYFEIDW	CDRRITLKG	NGKYVTAKKN	400
	human	351	GKYWTLTATG	GVQSTASSKN	AS-CYFDIEW	RDRIITLRAS	NGKFVTSKKN	400
	murine	351	GKYWTLTATG	GVQSTASTKN	AS-CYFDIEW	CDRRITLRAS	NGKFVTAKKN	400
	echinoid	351	DAKFWKTVA	GIQANGNSKD	QTDQCFSEY	NGNDMHVRAP	GGKYVSVRDN	400
	Drosophila	351	DRYWCLSAGG	GIQATGNRR	ADALFELIWH	GDGSLSFRA	NGKFLATKRS	400
			*	*			*	
	retinal fascin	401	GQLAAISDFV	GEDEEFTLKL	INRPILVLRG	LDGFVCHR-R	GSNQLDTNRS	450
	Xenopus	401	GQLSASVDTP	GETELFLMKL	INRPLIVLRG	EHCFIGCR-K	MTGTLDNSRS	450
	human	401	GQLAASVETA	GDSEFLMKL	INRPIIVFRG	EHCFIGCR-K	VTGTLDANRS	450
	murine	401	GHVAASVETA	GDSEFLMKL	INRPIIAFRG	EHCFIGCR-K	VTGTLDANRS	450
	echinoid	401	GHLFLQDSPK	D----FIFRL	LNRPKLVLKC	PHGFVGMK-E	GKAEVACNRS	450
	Drosophila	401	GHLFATSESI	EETAKFYFYL	INRPILVLKC	EQGFVGYRTP	GNLKLKCNKA	450
			*	*	*	**	*	
	retinal fascin	451	VYDVHLS-F	SDGAYQIRGR	GGGFWHTGSH	GSVCSGGERA	EDFLFFFRER	500
	Xenopus	451	IYDVFELE-F	NDGAYSLKDA	TGKYWTVGSD	MSVTSRCHPG	WS--LEFCDY	500
	human	451	SYDVQFLE-F	NDGAYNIKDS	TGKYWTVGSD	SAVTSSGDTF	VDFFFLEFCDY	500
	murine	451	SYDVQFLE-F	NDGAYNIKDS	TGKYWTVGSD	SSVTSSSDTP	VDFFFLEFCDY	500
	echinoid	451	NFDVFTVT-Y	KEGGYTIQDS	CGKYWSCDDS	SRIVLGEAAG	-TFFFEFHLE	500
	Drosophila	451	TYETILVERA	QKGLVHLKAH	SGKYWRIEGE	SISVDADAPS	DGFFLELREP	500
			*	*	*		*	
	retinal fascin	501	GRLAIRARS-	GKYLRCGASG	LLRADADAPA	GVALWEY...	.....	550
	Xenopus	501	NKVAIQKV-N	GLYLGKDHAG	VLKANAENID	STTLWEY...	.....	550
	human	501	NKVAI-KV-G	GRYLGKDHAG	VLKASAETVD	PASLWEY...	.....	550
	murine	501	NKVAL-KV-G	GRYLGKDHAG	VLKACAETID	PASLWEY...	.....	550
	echinoid	501	SKFAIRAESN	GMLIKGEQSG	LFTANGSEVS	KDTLWEF...	.....	550
	Drosophila	501	TRICIRSQ-Q	GKYLKATKNG	AFKLLDDGTD	SATQWEF...	.....	550
			*	*		**		

Fig. 1. (Continued).

**C** GTCCTGCTTTGCTACGGCCATCTCCCCGGCAGAGCTGTGGACCGTACACCTGGCCATCCACCCACAGGCTCACTTGCTGA 80  
 GTGTGAGCCGCCGGCGCTACGTGCACCTGTGCCTTCAGGAGGACGAGATGGCGGCAGATGGTGACATGCCGTGGGGTGTG 160  
 GACGCACTGCTCACCTCATTTCAGAGCAGGCGGTACTGCCTCAAGTCTTATGACAGCCGTACCTTCGAAGTGATGG 240  
 CCGCCTTGCTCTGGGAGCCTGAAGCCCATGCCTGTCTACACACTGGAGTTCAAGGCAGGCAAGCTGGCCTTCAAGGACTGTG 320  
 ACGGCCGATACCTGGCACCTGTGGGGCCTGCCGGCAGCTTAAGGCTGGCCGAAACACGAGGCCCGGCAAGGATGAACTC 400  
 ATCGACCTGGAGCAGAGTCACCCACAGGTGGTACTGGTAGCTGCCAACCACCGCTACGTCTCTGTGAGGCAAGGAATCAA 480  
 TGTCTCAGCC 490

Fig. 1. (Continued).

### 3. Results

#### 3.1. Cloning of retinal fascin

In order to isolate retina-specific genes, we performed three different methods; differential display RT-PCR between retina and brain mRNA; subtraction hybridization of lambda ZAP cDNA libraries prepared from rat retina and brain; sequencing of 100 independent clones randomly picked up from a bovine retina cDNA library. We have selected several candidate clones from each methods. Northern blot screening revealed one of the clones (R4) randomly picked up from a bovine retina cDNA library showed an eye-specific expression pattern. By screening of a bovine retina cDNA library with R4 probe, we isolated eight positive clones. Restriction enzyme mapping and partial sequencing indicated that these clones encode a single protein. The largest clone contains a cDNA insert of 1589 base pair with an open reading frame encoding a 492 amino acid protein with a calculated relative molecular mass of 55 070 Da. The nucleotide sequence and deduced amino acid sequence are shown in Fig. 1A. There is no N-terminal signal sequence. Hydropathy analysis of the primary amino acid sequence of the predicted protein shows absence of transmembrane domains. Computer analysis showed that there are nine potential sites for protein kinase C-dependent phosphorylation (Thr-20, Ser-39, Ser-147, Thr-237, Ser-272, Thr-300, Thr-375, Thr-404, Ser-465), two potential sites for protein kinase A-dependent phosphorylation (Thr-304, Ser-399), and two tyrosine kinase-dependent phosphorylation sites (Tyr-193, Tyr-228). Sequence analysis indicates that the deduced protein has 55% amino acid identity with murine fascin, 57% with human fascin, 52% with *Xenopus* fascin, 40% with *Drosophila* singed gene, 36% with echinoid fascin and shares most of the conserved motifs and conserved amino acids observed in the family (Fig. 1B). These findings indicate that retinal fascin is a novel member of fascin gene family.

#### 3.2. Northern blot analysis

Since rat is more convenient for analyzing the distribution of retinal fascin, we performed PCR to obtain rat retinal fascin. Rat retinal fascin cDNA had been partially cloned and sequenced by using the reverse transcription-polymerase chain reaction (RT-PCR) method. The degenerate oligonucleotide primers used in this reaction were designed from bovine fascin. The 5' primer [5'-TT(C/T)GGTGGCACCGA(A/G)GA(C/T)CA(A/G)(C/T)T-3'] corresponded to bp 367–389 of bovine retinal fascin sequence, and the 3' primer [5'-

TGATCCAG(T/C)TC(T/C)TC(G/A)TC(T/C)TG(G/A)TT-3'] corresponded to bp 880–902. We obtained a PCR fragment (Fig. 1C), which showed about 90% amino acid identity with bovine fascin, indicating that this clone is a partial fragment of rat retinal fascin. Northern blot analysis using rat retinal fascin as a probe revealed that a major band of approximately 1.5 kilobases and several minor bands were observed only in the eye but not in other tissues such as brain, lung, heart, liver, spleen, testis and skeletal muscle, suggesting that retinal fascin is specifically expressed in the eye and there are several splicing variants (Fig. 2).

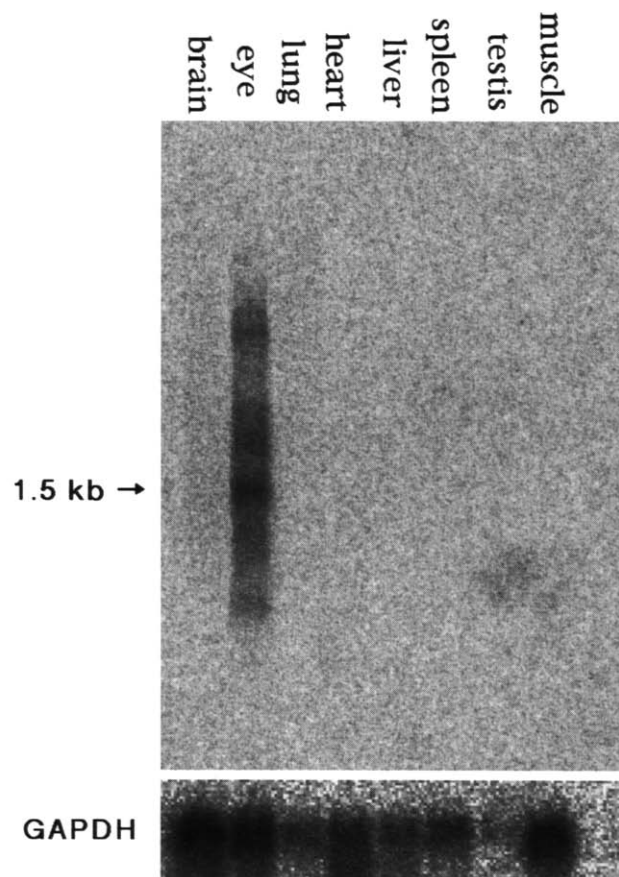


Fig. 2. Northern blot analysis of retinal fascin mRNA. 3 µg of mRNA from brain, eye, lung, heart, liver, spleen, testis and skeletal muscle.

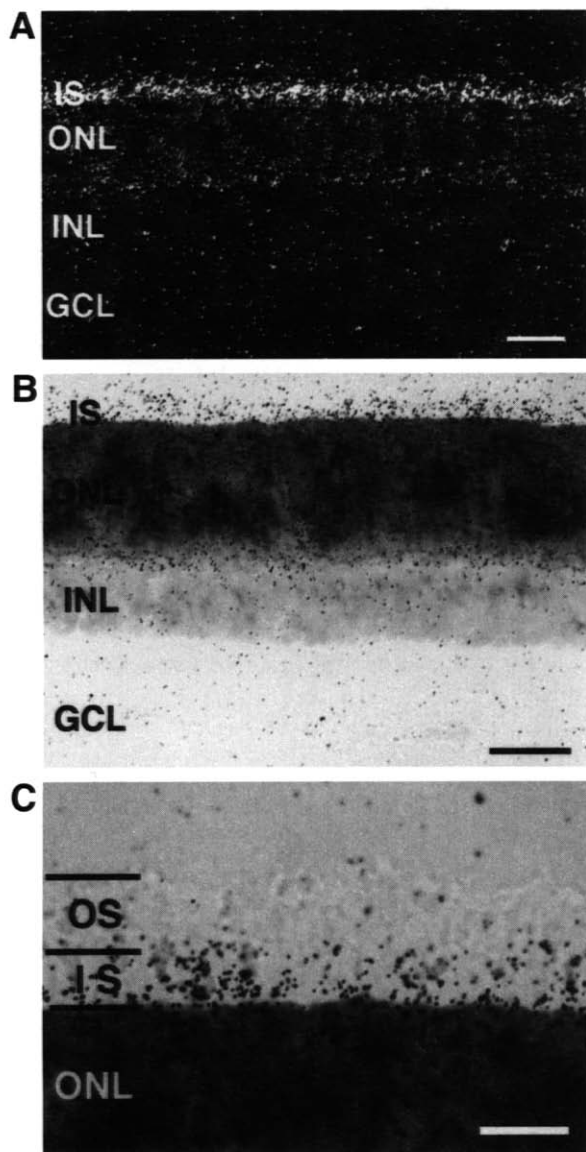


Fig. 3. Expression of retinal fascin mRNA in the adult rat retina. A: Dark-field photomicrograph. B: Bright-field photomicrograph. C: Bright-field photomicrograph of the photoreceptor cells. IS, inner segment; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. A: Bar = 20  $\mu$ m. B, C: Bar = 50  $\mu$ m.

### 3.3. In situ hybridization

To determine the more detailed distribution of retinal fascin in the rat retina, we performed in situ hybridization using a 35S-labeled cRNA probe. The specificity of the hybridization signals was determined by hybridizing consecutive sections with either antisense or sense probe. No specific signal was detected in sections hybridized with sense probe. In the rat retina, intense hybridization signals for retinal fascin mRNA were found in the inner segment of the photoreceptor cell layer (Fig. 3A and B). In addition, weak hybridization signals were detected in the outer nuclear layer (ONL) (Fig. 3A and B). At the high-power magnification bright field microscopic level, the signals for retinal fascin mRNA were concentrated in the inner segment (IS) of the retina (Fig. 3C), indicating that retinal fascin was exclusively expressed in photoreceptor cells. Similar photoreceptor cell-specific expression pat-

tern was also observed in the bovine retina by in situ hybridization with bovine retina fascin cRNA probe (data not shown).

### 4. Discussion

We describe the isolation and characterization of a novel cDNA which is exclusively expressed in the photoreceptor cells. Sequence analysis reveals that it is a novel member of fascin gene family, actin-bundling proteins. Thus, we named it retinal fascin. To date, members of fascin gene family have been cloned from sea urchin [4], *Drosophila* [22], *Xenopus* [7], mouse [6] and human [5]. They share three conserved regions [1]. The largest region is a 28 residue block near the N-terminus, 15 residues of which are invariant with the remainder being largely conservative substitutions. Retinal fascin has also the same region, in which the 15 conserved amino acids are completely identical. The other two regions are amino acids ETFQLE in the middle of the sequence and GKYW near the C-terminus. In these regions, retinal fascins also showed 50% identity. As a whole, retinal fascin shares 92% of the conserved amino acids in the family, indicating that it is a member of the family. In addition, although amino acid sequences showed 95% identity between human fascin and murine fascin, bovine retinal fascin shares only 57% amino acid identity with human fascin and 55% identity with murine fascin, revealing that retinal fascin is not a bovine homolog of human fascin and murine fascin, but a novel member of fascin gene family.

Mutation analysis of *Drosophila* fascin has shown that substitution glycine 409 with glutamic acid results in partial inactivation of fascin, and that substitution serine 289 with asparagine almost completely inactivates fascin in vivo [23]. These two amino acids are well conserved in the fascin family, and retinal fascin also has both two amino acids, suggesting that these regions are also functionally important in retinal fascin. Yamakita et al. has reported that phosphorylation by protein kinase C activator, TPA, greatly reduced the actin binding ability of human fascin, and discussed the physiological significance of phosphorylation of fascin in microfilament organization and cell motility [24]. They have identified one of the sites of human fascin phosphorylation as Ser-39. The site is well conserved among many fascins, including human, mouse *Xenopus*, and *Drosophila*. Retinal fascin also shares the corresponding Ser-39. In addition, computer analysis showed that retinal fascin may contain many putative phosphorylation sites for protein kinase A, protein kinase C, and tyrosine kinase, suggesting that retinal fascin is also regulated by phosphorylation.

The functions of fascins have been well characterized mainly in invertebrates. Fascins are thought to act as actin-bundling proteins in many structures, such as microspikes and stress fibers of fibroblasts, filopodia of coelomocytes, bristles and nurse cells of *Drosophila* [1]. Reconstitution experiments has shown that vertebrate and invertebrate fascin-actin bundles have different structures. The echinoid and singed bundles have an 11 nm transverse periodicity which is perpendicular to the long axis of the bundle, while murine bundles contain a transverse repeat every 36 nm which lies at a 60° angle to the bundle axis [1]. These differences in packing are considered to be due to fascin, not actin, raising the possibility that different actin bundles might require their own fascins.

In photoreceptor cells, actin is known to localize in the inner segment [8–12], synaptic region of rods [11–13], the connection between rod inner and outer segments [10,11], the lip of forming rod outer segment discs [10,14–16], and the basal end of the rod outer segment axis [15,17], and seems to be responsible for many functions, such as regulation of outer segment disk morphogenesis [18] and photoreceptor shortening during light or dark adaptation in lower vertebrates [19,20]. For example, in the presence of cytochalasin D, a drug that disrupts actin filaments, new disks are produced, but the new disks are fewer, larger and profoundly disordered in shape [18]. It has been, thus, speculated that actin and actin-associated proteins at the inner segment outer segment junction may be the key regulatory component of disk morphogenesis. Retinal fascin mRNA is exclusively expressed in the photoreceptor cells. Thus, it is quite possible that retinal fascin is also involved in disk morphogenesis.

In addition, although we did not show the existence of retinal fascin homologues in lower vertebrates, it is noteworthy that actin-dependent contraction in Teleost retinal rods depends on the intracellular concentration of cAMP [20]. The author discussed that cAMP might activate rod contraction by phosphorylation of some components of the contractile machinery. As mentioned above, retinal fascin shares two putative protein kinase A-dependent phosphorylation sites. Thus, it is quite possible that retinal fascin homologues in lower vertebrates might be involved in photoreceptor shortening during light or dark adaptation. Further studies using the specific antibody against retinal fascin is needed to know where in the photoreceptor cells it acts, and in which mechanisms it is involved.

## References

- [1] Edwards, R.A. and Bryan, J. (1995) *Cell Motil. Cytoskeleton* 32, 1–9.
- [2] Kane, R.E. (1975) *J. Cell Biol.* 66, 305–315.
- [3] Kane, R.E. (1976) *J. Cell Biol.* 71, 704–714.
- [4] Bryan, J., Edwards, R., Matsudaira, P., Otto, J. and Wulfskuhle, J. (1993) *Proc. Natl. Acad. Sci. USA* 90, 9115–9119.
- [5] Duh, F.M., Latif, F., Weng, Y., Geil, L., Modi, W., Stackhouse, T., Matsumura, F., Duan, D.R., Linehan, W.M., Lerman, M.I. and Gnarr, J.R. (1994) *DNA Cell Biol.* 13, 821–827.
- [6] Edwards, R.A., Herrera-Sosa, H., Otto, J. and Bryan, J. (1995) *J. Biol. Chem.* 270, 10764–10770.
- [7] Holthuis, J.C.M., Schoonderwoert, V.T.G. and Martens, G.J.M. (1994) *Biochim. Biophys. Acta* 1219, 184–188.
- [8] O'Connor, P. and Burnside, B. (1981) *J. Cell Biol.* 89, 517–524.
- [9] Drenckhahn, D. and Kaiser, H.W. (1983) *Eur. J. Cell Biol.* 31, 235–240.
- [10] Chaitin, M.H., Schneider, B.G., Hall, M.O. and Papermaster, D.S. (1984) *J. Cell Biol.* 99, 239–247.
- [11] Vaughan, D.K. and Fisher, S.K. (1987) *Exp. Eye Res.* 44, 393–406.
- [12] Woodford, B.J. and Blanks, J.C. (1989) *Cell Tissue Res.* 256, 495–505.
- [13] Drenckhahn, D. and Wagner, H.J. (1985) *Eur. J. Cell Biol.* 37, 156–168.
- [14] Chaitin, M.H. and Burnside, B. (1989) *Invest. Ophthalmol. Vis. Sci.* 30, 2461–2469.
- [15] Arikawa, K. and Williams, D.S. (1989) *J. Comp. Neurol.* 288, 640–646.
- [16] Chaitin, M.H. and Bok, D. (1986) *Invest. Ophthalmol. Vis. Sci.* 27, 1764–1767.
- [17] Roof, D.J. and Applebury, M.L. (1984) *J. Cell Biol.* 99, 114a.
- [18] Williams, D.S., Linberg, K.A., Vaughan, D.K., Fariss, R.N. and Fisher, S.K. (1988) *J. Comp. Neurol.* 272, 161–176.
- [19] Burnside, B. (1976) *Exp. Eye Res.* 23, 257–275.
- [20] O'Connor, P. and Burnside, B. (1982) *J. Cell Biol.* 95, 445–452.
- [21] Morimura, H., Shimada, H., Otori, Y., Saishin, Y., Yamauchi, A., Minami, Y., Inoue, K., Ishimoto, I., Tano, Y. and Tohyama, M. (1997) *Mol. Brain Res.* 44, 253–261.
- [22] Paterson, J. and O'Hare, K. (1991) *Genetics* 129, 1073–1084.
- [23] Cant, K. and Cooley, L. (1996) *Genetics* 143, 249–258.
- [24] Yamakita, Y., Ono, S., Matsumura, F. and Yamashiro, S. (1996) *J. Biol. Chem.* 271, 12632–12638.